Invited Review

Early Life Origins of Obesity: Role of Hypothalamic Programming

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ABSTRACT

The incidence of obesity is increasing at an alarming rate and this worldwide epidemic represents an ominous predictor of increases in diseases such as type 2 diabetes and metabolic syndrome. Epidemiological and animals studies suggest that maternal obesity and alterations in postnatal nutrition are associated with increased risks for obesity, hypertension, and type 2 diabetes in the offspring. Furthermore, there is also growing appreciation that developmental programming of neuroendocrine systems by the perinatal environment represents a possible cause for these diseases. This review article provides a synthesis of recent evidence concerning the actions of perinatal hormones and nutrition in programming the development and organization of hypothalamic circuits that regulate body weight and energy balance. Particular attention is given to the neurodevelopmental actions of insulin and leptin. J Pediatr Gastroenterol Nutr 48:S31–S38, 2009. Key Words: Development—Hormones—Hypothalamus—Leptin—Programming. © 2009 by European Society for Pediatric Gastroenterology, Hepatology, and Nutrition and North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition

A principal goal of brain development is to produce the necessary neural architecture for integrating information from the external environment with internal cues that reflect important aspects of an animal’s physiological state. This integration allows the elaboration of adaptive behavioral and physiological responses that are essential for survival. However, disorders can arise when an individual is confronted with environmental conditions that differ markedly from those present during perinatal development. For example, epidemiological evidence has indicated that alterations in perinatal nutrition could predispose an individual toward obesity and other associated diseases such as type 2 diabetes, particularly in an environment with high availability of energy dense foods. Paradoxically, both maternal obesity and maternal energy deprivation during pregnancy may increase the incidence of obesity and type 2 diabetes in the offspring (1–4). Mothers who are obese or have type 2 diabetes during pregnancy also have an increased incidence of obese progeny. Similarly, maternal malnutrition during gestation produces offspring obesity and diabetes (1,2). Data from a variety of animal models have supported a link between the perinatal nutritional environment and the programming of energy balance “set points.” Interestingly, both energy restriction and overfeeding could cause lasting perturbations in energy balance (1,2,5). In this review, we will attempt to examine these observations within the neurobiological aspects of hypothalamic programming.

NEURAL CONTROL OF ENERGY BALANCE

Signals Communicating Nutrient Availability in the Environment to the Developing Brain

Peripheral hormones represent important signals that regulate adiposity as well as central nervous system (CNS) circuits that control food intake. The best-characterized hormonal signals of adiposity are insulin and leptin (Fig. 1) (6). Insulin is secreted by the Islets of Langerhans in the pancreas to promote energy storage, and increased circulating insulin is observed in response to nutrient repletion and in states of obesity. Insulin receptors are expressed in the CNS (7) and injections of small amounts of insulin into the brain of insulin-deficient animals can eliminate hyperphagia (8). Deletion of the insulin receptor from the CNS
resulted in obesity and insulin resistance (9), thus further adding support to the importance of insulin action in appetite regulation by the brain. Insulin levels are elevated and known to mediate compensatory responses (such as macrosomia) in the offspring of diabetic mothers (10).

Leptin, a hormone secreted by fat cells, is a crucial signal of body energy stores and acts to downregulate feeding behavior and promote energy expenditure through a variety of neural and endocrine mechanisms. These include regulation of the autonomic nervous system and the synthesis of thyroid hormones (11). Thus, mice lacking leptin (Lep<sup>ob</sup>/Lep<sup>ob</sup> mice) are obese, diabetic, cold intolerant, and hypoactive (12,13). Similarly, mutations that affect the long form of the leptin receptor (Lep<sup>Rb</sup>) or its downstream signaling pathways result in a diabetic phenotype and infertility (14–17). Importantly, this receptor is highly expressed in regions of the CNS involved in energy balance, particularly the hypothalamus (18), and leptin acts directly on the CNS to mediate most of its action (19–21). Thus, leptin and insulin are particularly well suited to communicate nutrient availability in the environment to the hypothalamus during development.

**FIG. 1.** Organization of hypothalamic circuits regulating energy balance. These simplified schematic diagrams illustrate the possible routes and the neuronal populations relaying hormonal and nutrient signals from the periphery to the brain. Two distinct populations of neurons in the arcuate nucleus (ARH)—one coexpressing neuropeptide Y (NPY) and agouti-related protein (AgRP) and the other containing proopiomelanocortin (POMC)-derived peptides and cocaine- and amphetamine-regulated transcript (CART)—represent major routes for the regulation of body weight by peripheral hormones such as leptin. These neurons send direct projections to discrete populations of neurons located in the dorsomedial nuclei of the hypothalamus (DMH) and paraventricular nuclei of the hypothalamus (PVH) and in the lateral hypothalamic area (LHA). Each of these regions plays a major role in the control of ingestive behavior. Projections originating from the DMH and the ventromedial nucleus of the hypothalamus (VMH) and projecting to the PVH also represent important routes for the action of leptin at the hypothalamic level. me, median eminence; V3, third ventricle. The schematics have been modified with permission (6).
Hypothalamic Circuits Controlling Energy Balance

The hypothalamus is well known to regulate feeding and energy balance. Neurons in the arcuate nucleus of the hypothalamus (ARH) play an important role in this regulation. The ARH resides above the median eminence and shares connections with circumventricular organs, making it appropriate to receive and integrate signals from peripheral hormones such as leptin and insulin (Fig. 1). The ARH has long been associated with obesity (22), and it contains numerous leptin-sensitive neurons (18,23–26). Moreover, recent genetic studies have specifically demonstrated the importance of leptin receptor signaling in ARH neurons. Restoring ARH neurons’ leptin receptor signaling in leptin-deficient mice ameliorated body weight gain by reducing food intake and decreasing adipose tissue mass (21,27). These data indicate that the ARH is an important site of action for the central regulatory effects of leptin on energy balance. The ARH contains 2 populations of neurons that play particularly important roles in distributing leptin signals centrally. One subpopulation of ARH neurons coexpresses neuropeptide Y (NPY) and agouti-related peptide (AgRP) and acts as a major orexigenic signal (ie, promotes feeding). A separate subpopulation of ARH neurons expresses proopiomelanocortin (POMC)-derived peptides, such as alpha-melanocyte-stimulating hormone, and represents an important anorectic regulator (ie, inhibits feeding). These anatomically distinct populations of ARH neurons provide overlapping projections to other key parts of the hypothalamus that are implicated in the control of feeding. These hypothalamic parts include the paraventricular nuclei of the hypothalamus (PVH) and dorsomedial nuclei of the hypothalamus (DMH), as well as the lateral hypothalamic area (LHA).

In addition to playing an important role in regulating food intake and body weight, a number of studies have suggested that the hypothalamus is a key component of peripheral glucose homeostasis. Infusion of insulin into the medio-basal hypothalamus (a region comprising the ARH and ventromedial nucleus of the hypothalamus, VMH) reduced hepatic glucose homeostasis by increasing hepatic insulin sensitivity (28). Furthermore, downregulation of insulin receptor signaling in the medio-basal hypothalamus induced insulin resistance in rats (28–30). Altogether these data show that hypothalamic insulin signaling is important for the regulation of glucose homeostasis. Recent data have also indicated that most of the effects of leptin on glucose homeostasis are mediated by its effects on the hypothalamus. Restoration of functional leptin receptors exclusively in the ARH of leptin-receptor-deficient animals significantly improved glucose homeostasis and insulin sensitivity (27,31).

Thus, the hypothalamus appears to play a major role in the regulation of energy balance and glucose homeostasis and its core circuitry appears to mediate many of the metabolic effects of leptin and insulin (Fig. 1).

DEVELOPMENT OF HYPOTHALAMIC FEEDING CIRCUITS

Rodent Studies

Hypothalamic development is initiated by cell proliferation in the neuroepithelium of the third ventricle. This involves generation of neuronal progenitors that ultimately produce postmitotic neurons (Fig. 2). These postmitotic neurons migrate to their appropriate location in various parts of the hypothalamus. Neuronal birth-dating studies in rats revealed that ARH neurons are generated between embryonic day (E) 12 and E17, VMH neurons between E13 and E16, DMH neurons between E12 and E16, PVH neurons between E13 and E15, and LHA neurons between E12 and E14 (see 32). Although hypothalamic neuronal proliferation occurs primarily during mid-gestation, the development of neural projections from these neurons to their downstream target sites is initiated primarily postnatally (Fig. 2). Axonal tract tracing experiments performed in mice revealed that ARH projections reached their target nuclei within distinct temporal domains; innervation of the DMH occurred first on postnatal day (P) 6, followed by innervation of the PVH on P8-P10 (33). Projections to the LHA were established later in P12 (33). Immunohistochemical studies in rats showed that development of axonal projections from ARH NPY/AgRP neurons follow similar temporal domains (34). It is also interesting to note that a significant proportion of NPY could be produced by neurons in the DMH and LHA during the first weeks of postnatal life, in addition to what is already produced by ARH neurons (35). However, the precise role and function of these transient populations of NPY neurons remain unclear. Nevertheless, we should note that NPY is permanently induced in the DMH of obese

FIG. 2. Critical periods of hypothalamic development in rodents. Schematic drawings summarizing major neurobiological events governing hypothalamic development. The growth of the hypothalamus after organogenesis can be described as occurring in 2 major phases: a phase of neuronal proliferation (neurogenesis) that takes place between embryonic days 12 and 16, followed by a phase of axonal extension that occurs between postnatal days 6 and 12.
animals (specifically in the melanocortin 4 [MC4] receptor knockout and the lethal yellow [A(y)] mice), raising the possibility that this ectopic population of NPY neurons may play an important role in the development of obesity (36). Together, these data reveal the existence of 2 major critical periods (ie, mid-gestation and early postnatal life) during which alterations in the intrauterine environment may affect hypothalamic neurogenesis and/or axonal outgrowth and, therefore, will have long-term consequences on nutrition and metabolism.

Human and Non-human Primate Studies

Limited information is available on how the hypothalamus develops in humans. Much of what we know about the development of hypothalamic neural pathways in primates has been inferred from studies in nonhuman primates. Hypothalamic neurogenesis in these animals occurs in the first quarter of gestation (37,38). Limited reports on human fetal chemosignature and cytoarchitecture have suggested that early hypothalamic neurogenesis is limited to the 9th and 10th weeks of gestation (39–43). Although many of the hypothalamic feeding circuits develop during the first 2 weeks of life in rodents, these circuits appear to develop in utero in primates, including humans. In Japanese macaques, NPY/AgRP fibers innervate the PVH as early as gestational day 100 (ie, late second trimester of gestation) and a mature pattern of projections is apparent at gestational day 170 (44). Similarly, in human fetuses, NPY immunoreactive fibers are detected in the ARH and in the PVH as early as at 21 weeks of gestation (45). Thus, development of neural projection in humans occurs significantly later than neurogenesis. Whether the same developmental factors influence both neuronal proliferation and axonal extension is unknown. However, it is notable that these 2 developmental events occur during distinct temporal periods.

Developmental Regulation of Leptin’s Action on Metabolism

In addition to its effects on appetite regulation in adults, leptin also regulates appetite-related neuropeptides in the hypothalamus during early development. Administration of leptin to P10 rats increased suppressor of cytokine signaling 3 (SOCS-3) and POMC mRNA levels, but decreased NPY mRNA levels in the ARH (46). Moreover, chronic neonatal leptin administration downregulated all subtypes of leptin receptor mRNA and increased corticotropin-releasing factor receptor-2 mRNA levels in the VMH (46). Furthermore, leptin induced cFos expression in ARH neurons (specifically in POMC neurons) as early as P6 (33). However, these transcriptional changes were not matched by a corresponding reduction in food intake in neonatal mice, because administration of leptin in lean or Lep(ob)Lep(ob) mice did not affect milk/food intake, oxygen consumption, body weight, or adiposity until after weaning (46,47). Therefore, despite its regulatory action on hypothalamic neuropeptide expression, leptin does not appear to regulate food intake during early development. This decreased anorectic action of leptin before weaning may help the animals maximize food intake to support growth and to maintain high thermoregulatory metabolic rates to optimize survival until weaning.

PERINATAL FACTORS INFLUENCING DEVELOPMENT OF HYPOTHALAMIC FEEDING PATHWAYS

As noted above, a plethora of data from rodent to human studies have suggested that nutritional status during early development affects the later metabolic fate of the organism. Insulin and leptin thus likely represent the hormonal mediators for these environmental nutrient sensing systems that control this program (Fig. 3).

Leptin

It is now clear that in addition to playing an important role in the regulation of energy balance and neuroendocrine functions in mature animals, leptin also acts early in life as a developmental signal that promotes the formation of metabolic pathways. Elevated leptin levels are found particularly during the first 2 weeks of life in rodents (48–50) at a time when leptin is largely ineffective in altering body weight or food intake. Limited information is available on the origin of the neonatal leptin surge. It is probable that neonatal leptin is produced, at least in part, by fetal adipose tissue, as revealed by the elevated neonatal leptin mRNA expression in white and brown adipose tissues that mirrors the circulating hormone concentrations (50). Alternatively, perinatal leptin may also be produced by other organs such as the stomach (51). Moreover, several studies have shown the important contribution of maternal milk to serum leptin levels in newborns (52,53). Interestingly, the neonatal leptin surge (48) appeared to coincide with the development of major hypothalamic feeding circuits (54). Neuroanatomical experiments further revealed that instead of regulating food intake and body weight, neonatal leptin is an important trophic factor for the development of hypothalamic circuits that control energy homeostasis. Injections of anterograde axonal tracers into the ARH of leptin-deficient mice demonstrated that leptin deficiency induced profound disruption in the formation of ARH circuits (55). The density of axons from arcuate nucleus neurons that innervate other hypothalamic sites involved in the control of energy homeostasis (such as the PVH, DMH, and LHA) is severely reduced in Lep(ob)Lep(ob) neonates and remains diminished throughout life (55).
Similar disruptions were observed in other animal models of leptin receptor deficiency, such as in Zucker rats (54). Both orexigenic (NPY/AgRP) and anorexigenic (POMC) projections appeared to be affected by leptin deficiency (55), suggesting a widespread developmental effect of leptin on arcuate neurons involved in the regulation of metabolism. In vitro experiments also revealed that leptin could act directly on ARH neurons to induce axonal outgrowth (55). Furthermore, leptin appeared to exert its effects on axonal formation primarily during a restricted postnatal period, because daily injections of P4 to P12 Lep ob/Lep ob mice with leptin rescued a normal pattern of innervation by arcuate neurons of the PVH. On the contrary, injections of the hormone in mature animals remained largely ineffective in restoring a normal pattern of ARH projections (55). These data indicate that there is a critical period for the neurodevelopmental actions of leptin that seems to be restricted to the first few weeks of life. The existence of a critical period for the developmental effects of leptin suggests that changes in leptin levels during key periods of hypothalamic development may induce long-lasting and potentially irreversible effects on metabolism in adults. Leptin levels are directly regulated by nutritional factors, thus this hormone is well positioned to participate in developmental responses to nutritional changes. In support of this hypothesis, recent data have indicated that an ill-timed neonatal leptin surge may cause lasting effects on metabolism. Using a mouse model of intra-uterine energy restriction, Yura et al (56) found that prenatal underfeeding resulted in an earlier leptin surge that was accompanied by deleterious effects on body weight regulation and glucose homeostasis. The premature leptin surge observed in the offspring of undernourished dams was also associated with a reduced anorectic effect of leptin and an altered hypothalamic response to leptin, as evidenced by a decreased leptin-induced cFos immunoreactivity in the PVH (56). Similarly, the blunted postnatal leptin surge, as induced by the administration of a specific leptin antagonist from P2 to P13, was associated with long-term leptin insensitivity and increased susceptibility to diet-induced obesity (DIO) in rats (57).

Taken together, these studies have suggested that the postnatal leptin surge is an important trophic factor for the development of hypothalamic feeding circuits and is critical for normal energy balance and hypothalamic regulation later in life.

**Insulin**

In addition to leptin, insulin also appears to exert important influences on the development of hypothalamic circuits that regulate energy homeostasis. Maternal injections of insulin between gestational day 15 and 20, a critical period for hypothalamic development, induced obesity in the offspring (58). The metabolic abnormalities observed in the offspring of insulin-injected dams...
were also accompanied by increased hypothalamic nor-
epinephrine levels (58) and increased density of norepi-
epinephrine-containing fibers innervating the PVH (59).
Similarly, maternal diabetes induced by streptozotocin
injections resulted in hyperinsulinism associated with
hypothalamic alterations such as decreased brain leptin
mRNA and protein expression (60), as well as altered
neuronal morphology in the arcuate nucleus in the fetus
(10). Furthermore, postnatal injections of insulin have
been associated with morphological changes in the VMH
(61). Together, these data suggest that changes in insulin
levels (specifically hyperinsulinism) during pregnancy
could induce alterations in hypothalamic organization
that may affect metabolism of the offspring later in life.

**Polygenic Obesity**

It is increasingly accepted that obesity results from a
combination of genetic and environmental factors. Diet-
induced obesity in rats is a useful model to study the pathogenesis of human obesity because DIO rats, like
humans, have a polygenic mode of inheritance. More-
over, these rats develop metabolic syndrome when a
moderate amount of fat is added to the diet (62,63).
One of the particular traits of DIO rats is that they exhibit
leptin resistance characterized by elevated serum leptin
and a decreased anorectic and thermogenic response
to exogenous leptin (63–65). The body of evidence
suggests that leptin resistance observed in DIO rats is
mediated by central leptin insensitivity. For example,
DIO rats have decreased expression of LepRb associated
with attenuated leptin receptor signaling in the hypo-
thalamus, particularly in the ARH (63,65,66). Interest-
ingly, this reduction in hypothalamic leptin sensitivity
occurred before the animals became obese and was
established during early postnatal life (67). The dimin-
ished responsiveness of hypothalamic neurons to leptin
appeared to impact the development of hypothalamic
circuits. Thus, the density of axons emanating from the
ARH and innervating the PVH appeared severely reduced in the progeny of DIO mothers compared with the
offspring of diet-resistant (DR) dams (67). Moreover,
ARH neurons derived from DIO rats were significantly
less responsive to the neurotrophic action of leptin than
ARH neurons in explants derived from DR rats (67).
Thus, polygenic obesity appears to induce the abnormal
organization of neural pathways involved in energy
homeostasis; this may be the result of the diminished
responsiveness of ARH neurons to the trophic actions of
leptin during critical periods of postnatal development.

**Perinatal Nutrition**

It has been known for decades that changes in perinatal
nutrition have long-term effects on metabolism (1,4,68).
Previous studies have suggested that neonatal nutrition
may also play an important role in the programming of
hypothalamic feeding systems (Fig. 3). Using an animal
model of divergent litter size, Plagemann et al (2)
demonstrated that animals that were raised in small litters
(3 pups per litter) showed increased body weight and
adiposity during adult life. These metabolic abnormal-
ities were associated with altered responsiveness of ARH
and VMH neurons to insulin and leptin. Although leptin
is a major stimulatory signal on VMH neurons in normal
animals, it mainly inhibits VMH neurons in rats that are
raised in small litters (69). Similarly, postnatal overnu-
trition induced a reduction in the inhibitory effect of
leptin on ARH neurons (70). Moreover, ARH neurons of
rats exposed to early postnatal overfeeding were less
inhibited by insulin when compared with controls (71).
Changes in postnatal nutrition also modified the response of
VMH neurons to orexigenic peptides (NPY and AgRP)
and of PVH neurons to anorexigenic neuropeptides
(aMSH) and cocaine- and amphetamine-regulated tran-
script (CART) (72–74). Taken together, these data
demonstrate that alteration in nutrition during critical periods
of postnatal development may induce permanent changes
in the responsiveness of hypothalamic neurons to hor-
monal and peptidergic cues.

Nutrition during prenatal life also appears to influence
the programming of hypothalamic appetite networks. Maternal protein restriction induced hypoinsulinemia
in the offspring and was associated with increased
NPY levels in the PVH and LHA at weaning (75). Importantly, exposure to high doses of NPY during early
postnatal development was linked to permanent changes
in food intake in adults (76). Similarly, increased
maternal nutrition in late pregnancy resulted in persistent
changes in the hypothalamic expression of appetite-regu-

ing genes in sheep (77). Offspring of dams fed with
40% excess nutrient intake in late pregnancy had a
permanent increase in hypothalamic POMC mRNA
expression when compared with that of control animals
(77). In addition to altering gene expression, maternal
overnutrition also affected central leptin sensitivity, as
demonstrated by the attenuated levels of leptin-induced
phosphorylation of the signal transducer and activator of
transcription 3 (pSTAT3, a key intracellular signaling
pathway of LepRb) in offspring born from dams fed with
a high-fat diet (78).

**CONCLUSIONS**

Previous studies during the past decade have indicated
that normal metabolic regulation during adulthood not
only requires a good matching of energy intake with
energy expenditure but also is influenced by optimal fetal
and postnatal environments. Although the mechanisms
underlying this metabolic imprinting require further
elucidation, the evidence accumulated to date indicates
that perinatal hormones (particularly insulin and leptin)
represent key signals that program CNS (hypothalamic) development and function and exert lasting effects on body weight regulation and glucose homeostasis. A better understanding of how these metabolic hormones exert their neurotrophic effects may open new avenues for understanding pre- and perinatally acquired predisposition to obesity and diabetes. Furthermore, a more detailed determination of whether hypothalamic misprogramming can be reversed, and the definition of the precise limits of the critical period for plasticity may provide new preventive and/or therapeutic opportunities.

REFERENCES


